L6 ANSWER 7 OF 15 MEDLINE on STN
ACCESSION NUMBER: 94334369 MEDLINE
DOCUMENT NUMBER: 94334369 PubMed ID: 8056829
TITLE: Immunolocalisation of collagenase in rabbit periosteal tissue explants and extraction of the enzyme. The effect of the cytokines IL-1 alpha and EGF.
AUTHOR: van der Zee E; Everts V; Hoeben K; Beertsen W
CORPORATE SOURCE: Department of Periodontology, Academic Centre for Dentistry Amsterdam (ACTA), The Netherlands.
CONTRACT NUMBER: NICHD N01-HD-6-2915 (NICHD)
SOURCE: JOURNAL OF CELL SCIENCE, (1994 Apr) 107 (Pt 4) 1047-53. Journal code: 0052457. ISSN: 0021-9533.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 19940920
Last Updated on STN: 20000303
Entered Medline: 19940913

AB The effect of interleukin-1 alpha (**IL-1** alpha) and murine epidermal growth factor (EGF) on incorporation of endogenously produced collagenase in the extracellular matrix of soft **connective tissue** was studied in an in **vitro** model system using periosteal explants obtained from rabbit calvariae. Immunohistochemical analysis indicated the highest level of collagenase in explants cultured for 72 hours with **IL-1** alpha in combination with EGF. Most enzyme appeared to be associated with the extracellular matrix, but labeling was also found in numerous fibroblast-like cells. Explants cultured in the presence of **IL-1** alpha alone contained less enzyme and in periostea **treated** without cytokines, or with EGF alone, only a faint label, if any, was seen. Freshly isolated, non-cultured periostea contained no detectable enzyme. Extraction of collagenase from periostea revealed that: (1) non-cultured periosteum did not contain detectable levels of enzyme. (2) The amount of total activatable enzyme synergistically increased (10-fold) under the influence of **IL-1** alpha and EGF, whereas **IL-1** alpha alone showed a 4-fold enhancement compared to control or EGF-incubated explants. (3) The latent fraction of the enzyme was synergistically increased (up to 100-fold or more) in periostea cultured in the presence of **IL-1** alpha + EGF (21.17 mU/explant versus 0.05 mU/explant in controls). (4) Active collagenase, on the other hand, appeared to be present in a relatively high concentration in explants cultured without cytokines (2.45 mU/explant versus 0.36 mU/explant in **IL-1** alpha + EGF-**treated** explants).(ABSTRACT TRUNCATED AT 250 WORDS)

=> D 1-15 ABS IBIB

L8 ANSWER 1 OF 15 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN DUPLICATE 1

AB Objective. To explore the role of reactive oxygen species (ROS) in the in **vitro** activation of skin fibroblasts from patients with systemic sclerosis (SSc). Methods. Fibroblasts were obtained from involved skin of patients with limited or diffuse SSc. Oxidative activity imaging in living cells was carried out using confocal microscopy. Levels of O(2)(-) and H(2)O(2) released from fibroblasts were estimated by the superoxide dismutase (SOD)-inhibitable cytochrome c reduction and homovanilic acid assays, respectively. To verify NADPH oxidase activation, the light membrane of fibroblasts was immunoblotted with an anti-p47(phox)-specific antibody. Fibroblasts were stimulated with various cytokines and growth factors to determine whether any of these factors modulate ROS generation. Cell proliferation was estimated by (3)H-thymidine incorporation. Northern blot analysis was used to study .alpha.1 and .alpha.2 type I collagen gene expression. Results. Unstimulated skin fibroblasts from SSc patients released more O(2)(-) and H(2)O(2) in **vitro** through the NADPH oxidase complex pathway than did normal fibroblasts, since incubation of SSc fibroblasts with diphenylene iodonium, a flavoprotein inhibitor, suppressed the generation of ROS. This suppression was not seen with rotenone, a mitochondrial oxidase inhibitor, or allopurinol, a xanthine oxidase inhibitor. Furthermore, the cytosolic component of NADPH oxidase, p47(phox), was translocated to the plasma membrane of resting SSc fibroblasts. A transient increase in ROS production was induced in normal but not in SSc fibroblasts by interleukin-1.beta. (**IL-1** .beta.), platelet-derived growth factor type BB (PDGF-BB), transforming growth factor .beta.1 (TGF.beta.1), and H(2)O(2). **Treatment** of normal and SSc fibroblasts with tumor necrosis factor .alpha. (TNF.alpha.), IL-2, IL-4, IL-6, IL-10, interferon-.alpha. (IFN.alpha.), IFN.gamma., granulocyte-macrophage colony-stimulating factor (GM-CSP), G-CSF, or **connective tissue** growth factor (CTGF) had no effect on ROS generation. Constitutive ROS production by SSc fibroblasts was not inhibited when these cells were **treated** with catalase, SOD, **IL-1** receptor antagonist, or antibodies blocking the effect of TGF.beta.1, PDGF-BB, and other agonists (IL-4, IL-6, TNF.alpha., CTGF). In contrast, **treatment** of SSc fibroblasts with the membrane-permeant antioxidant N-acetyl-L-Cysteine inhibited ROS production, and this was accompanied by decreased proliferation of these cells and down-regulation of .alpha.1(I) and .alpha.2(I) collagen messenger RNA. Conclusion. The constitutive intracellular production of ROS by SSc fibroblasts derives from the activation of an NADPH oxidase-like system and is essential to fibroblast proliferation and expression of type I collagen genes in SSc cells. Our results also exclude O(2)(-), H(2)O(2), **IL-1**.beta., TGF.beta.1, PDGF-BB, IL-4, IL-6, TNF.alpha., or CTGF as mediators of a positive, autocrine feedback mechanism of ROS generation.

ACCESSION NUMBER: 2001399539 EMBASE

TITLE: Oxidative stress in scleroderma: Maintenance of scleroderma fibroblast phenotype by the constitutive up-regulation of reactive oxygen species generation through the NADPH oxidase complex pathway.

AUTHOR: Sambo P.; Baroni S.S.; Luchetti M.; Paroncini P.; Dusi S.; Orlandini G.; Gabrielli A.

CORPORATE SOURCE: Dr. A. Gabrielli, Istituto di Clinica Medica Generale, Ematologia ed Immunologia Clinica, Via Tronto, 10, 60020 Ancona, Italy. a.gabrielli@popcsi.unian.it

SOURCE: Arthritis and Rheumatism, (2001) 44/11 (2653-2664).
Refs: 42
ISSN: 0004-3591 CODEN: ARHEAW

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 031 Arthritis and Rheumatism

LANGUAGE: English

SUMMARY LANGUAGE: English

L8 ANSWER 2 OF 15 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN

AB Background: Cyclosporine A (CsA) is a widely used immunosuppressant, with clinical applications ranging from organ transplants to chronic

inflammatory diseases. One of the side effects associated with CsA **treatment** is the development of gingival overgrowth. Exuberant growth of **connective tissue** within the periodontium can result from hyperactivity of resident fibroblasts. Fibroblasts are capable of secreting interleukin-6 (IL-6), which has been shown to enhance proliferation as well as collagen and glycosaminoglycan synthesis by these cells. We tested the hypothesis that one of the pathogenetic mechanisms underlying CsA-induced fibrosis is an enhanced IL-6 secretion by gingival fibroblasts (GF) in response to this drug. Methods: The ability of CsA to upregulate GF IL-6 secretion alone or in combination with bacterial challenge or other inflammatory cytokines was tested in an in **vitro** system. Fibroblast cultures were established from systemically healthy gingival tissue donors and were challenged with CsA in the absence or presence of bacteria, **IL-1**.beta., or tumor necrosis factor (TNF) .alpha. as co-stimulants. Nifedipine and phenytoin were also tested to further support findings with CsA. After 72 hours of incubation, culture supernatants were collected and analyzed for IL-6 content by ELISA. Results: We have shown that GF respond to CsA with an increase in IL-6 secretion. The magnitude of this response varies among cultures derived from different tissue donors. We have also demonstrated that GF IL-6 responses to bacterial challenge or TNF.alpha. are downregulated by CsA. However, CsA synergizes with **IL-1** .beta. to further upregulate IL-6 secretion, and this effect is shared by phenytoin and nifedipine. Conclusions: We conclude that one of the pathogenetic mechanisms underlying drug-induced gingival overgrowth may be enhanced secretion of IL-6 by GF in response to these medications. This is the first report on direct and indirect effects of gingival overgrowth-related medications on GF IL-6 metabolism. This work will lay the foundation for future studies directed towards the development of prevention or **treatment** modalities for gingival overgrowth based on blocking the fibrogenic activities of IL-6 at the cellular level.

ACCESSION NUMBER: 2000022498 EMBASE
TITLE: Regulation of gingival fibroblast interleukin-6 secretion by cyclosporine A.
AUTHOR: Morton R.S.; Dongari-Bagtzoglu A.I.
CORPORATE SOURCE: Dr. A.I. Dongari-Bagtzoglu, Columbia University, School of Dental and Oral Surgery, 630 W. 168th St., New York, NY 10032, United States
SOURCE: Journal of Periodontology, (1999) 70/12 (1464-1471).
Refs: 48
ISSN: 0022-3492 CODEN: JOPRAJ
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 011 Otorhinolaryngology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

L8 ANSWER 3 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2

AB Matrix metalloproteinase-3 (MMP-3), or stromelysin-1, is an enzyme responsible for the degradation of a wide range of extracellular matrix proteins. Increases in MMP-3 activity have been found in several chronic inflammatory diseases, and this increased activity is thought to be mediated by interleukin-1-beta (**IL-1**-beta). Because **IL-1**-beta has been strongly associated with inflammatory periodontal disease, the purpose of this in **vitro** study was to investigate the role of **IL-1**-beta on the regulation of MMP-3 levels in cells derived from the human periodontal ligament (PDL). Human PDL cell cultures were **treated** with **IL-1** -beta at varying concentrations (0.01-1.0 ng/ml) for 24 hours prior to analysis at either transcript or protein levels. Following the isolation of total RNA, the relative levels of MMP-3 mRNA were determined using reverse transcription-polymerase chain reaction (RT-PCR) with 32P-end-labeled primers. Immunocytochemical detection of MMP-3 protein was performed using polyclonal antibodies to human MMP-3. The results of RT-PCR analysis demonstrated a concentration-dependent increase in MMP-3 mRNA expression, with **IL-1**-beta **treatments** of 0.1 and 1.0 ng/ml significantly (P lt 0.01) increased over those cells

not **treated** with **IL-1**-beta. This increase in mRNA expression was paralleled by significant (P lt 0.001) changes at the protein level, with an average of 27.6% of the cells stained positive for MMP-3 following **IL-1**-beta **treatment** (1.0 ng/ml), compared with control cells showing no positive staining for MMP-3. In conclusion, the results of this study demonstrate that **IL-1**-beta upregulates MMP-3 in human PDL cells on both an mRNA and a protein level. These findings suggest possibly important roles for **IL-1**-beta and MMP-3 in both normal turnover and maintenance of the PDL and in the **connective tissue** degradation associated with periodontal disease.

ACCESSION NUMBER: 1997:342074 BIOSIS
DOCUMENT NUMBER: PREV199799641277
TITLE: Effects of interleukin-1-beta on matrix metalloproteinase-3 levels in human periodontal ligament cells.
AUTHOR(S): Nakaya, H.; Oates, T. W.; Hoang, A. M.; Kamoi, K.; Cochran, D. L. (1)
CORPORATE SOURCE: (1) Dep. Periodontics, UTHSCSA, 7703 Curl Dr., San Antonio, TX 78284 USA
SOURCE: Journal of Periodontology, (1997) Vol. 68, No. 6, pp. 517-523.
ISSN: 0022-3492.
DOCUMENT TYPE: Article
LANGUAGE: English

L8 ANSWER 4 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 3

AB Recent in **vitro** findings indicate that cytokines represent an important pathway of **connective tissue** destruction in human periodontitis. The biological effects of interleukin-1-beta (**IL-1**-beta) and interleukin-8 (IL-8) are relevant in this regard, and the objective of this study was to compare the levels of these molecules in gingival crevicular fluids (GCF) from patients with adult periodontitis (experimental group) and from individuals with clinically healthy gingiva (control group). GCF was collected for 30 seconds using a periopaper strip and the volume of the sample determined. Following elution of the fluid, assays for **IL-1**-beta and IL-8 were carried out by ELISA. The concentrations (ng/ml) of cytokines were calculated in the original volume of GCF on each strip. The total amounts (pg/site) of cytokines were expressed as the concentrations multiplied by volumes of GCF. The total amounts of **IL-1**-beta and IL-8 of the experimental group were significantly higher than the control group. The total amounts of both cytokines were markedly reduced following phase 1 periodontal **treatment**. The clinical parameters were positively related to the total amounts of **IL-1**-beta and IL-8. **IL-1**-beta concentrations and total amounts were also positively related to IL-8 suggesting that the GCF IL-8 levels are influenced by local **IL-1**-beta activities. These data indicate that the total amounts of **IL-1**-beta and IL-8 exhibited dynamic changes upon severity of periodontal disease. The levels of **IL-1**-beta and IL-8 in GCF are valuable in detecting the inflammation of periodontal tissue.

ACCESSION NUMBER: 1995:530219 BIOSIS
DOCUMENT NUMBER: PREV199598544519
TITLE: Levels of interleukin-1-beta and interleukin-8 in gingival crevicular fluids in adult periodontitis.
AUTHOR(S): Tsai, Chi-Cheng (1); Ho, Yea-Pyng; Chen, Ching-Charng
CORPORATE SOURCE: (1) Sch. Dent., Kaohsiung Med. Coll., No. 100, Shih-Chuan 1st Road, Kaohsiung City 807 Taiwan
SOURCE: Journal of Periodontology, (1995) Vol. 66, No. 10, pp. 852-859.
ISSN: 0022-3492.
DOCUMENT TYPE: Article
LANGUAGE: English

L8 ANSWER 5 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 4

AB Investigative attempts to identify novel therapy for inflammatory **connective tissue** diseases continue to evolve. Amiprilose hydrochloride (amiprilose HCl) is a synthetic carbohydrate

shown to have anti-inflammatory effects in animal models of inflammatory arthritis and in a multicenter clinical trial. Interleukin-1 (**IL-1**) is an important mediator of immune regulation, inflammation and joint destruction in arthritis. In the present study, the effects of amiprilose HCl on **IL-1** activity, production and receptor distribution were investigated. Drug effects on IL-2 Production and receptor distribution on lymphocytes were also explored. Potential regulation of **IL-1** activity was determined by monitoring the effects of amiprilose HCl on **IL-1** stimulated proliferation of murine thymocytes and human synovial cells. Inhibitory effects on **IL-1**-beta and IL-2 production by stimulated human peripheral blood monocytes were measured by ELISA and lymphocyte **IL-1**-beta and IL-2 receptor distribution were analyzed by flow cytometry. The results from in **vitro** studies demonstrated that low concentrations of amiprilose HCl (1-100 mu-g/ml) stimulated thymocyte proliferation and enhanced the proliferative response of **IL-1** stimulated human synovial fibroblasts. **IL-1**-beta production in cultures of human peripheral blood monocytes was significantly decreased after exposure of the cultures to varying doses of amiprilose HCl as determined by ELISA. Exposure of mitogen activated human peripheral blood lymphocytes to amiprilose HCl resulted in decreased IL-2 production at high concentrations of drug as compared to control. However, at doses of amiprilose HCl previously found to stimulate thymocyte proliferation (1-10 mu-g/ml), increased levels of culture supernatant IL-2 were observed. No amiprilose HCl mediated changes in lymphocyte **IL-1**-beta or IL-2 receptor expression were observed. The regulatory effects of amiprilose HCl on cytokines support the potential of this drug as a therapeutic agent for the **treatment** of inflammatory arthritis.

ACCESSION NUMBER: 1995:458978 BIOSIS
DOCUMENT NUMBER: PREV199598473278
TITLE: Immunoregulatory effects of a synthetic monosaccharide.
AUTHOR(S): Chang, D. M.
CORPORATE SOURCE: Rheumatol./Immunol./Allergy, Tri-Serv. General Hosp., Natl. Defense Med. Cent., Taipei Taiwan
SOURCE: Immunopharmacology and Immunotoxicology, (1995) Vol. 17, No. 3, pp. 437-450.
ISSN: 0892-3973.
DOCUMENT TYPE: Article
LANGUAGE: English

L8 ANSWER 6 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 5

AB Chronic inflammation and degradation of **connective tissue** in the course of periodontitis are maintained by bacterial products such as lipopolysaccharides (LPS), which probably act via inflammation mediators, e.g. cytokines. We investigated the effects of lipopolysaccharide (LPS) from E. coli and mouse recombinant interleukin 1-alpha (mrIL-1) on chondrogenesis, endochondral mineralization, matrix metalloproteinase activation and matrix degradation in **vitro** using cartilage organoid cultures. Mesenchymal cells of limb buds from mouse embryos (day 12) were grown at high density on a membrane filter at the medium/air interphase for 14 days. Chondrogenesis occurred during the first 6 days of culture. Endochondral mineralization took place upon addition of 5 mM beta-glycerophosphate from day 7 to 14. **Treatment** of the cultures with LPS and mrIL-1 on days 2 to 14 and during mineralization on days 7 to 14 resulted in a marked decrease of types I and II collagen, matrix mineralization and proteoglycan content. In the medium, proteoglycan content and metalloproteinase activity were enhanced. LPS induced **IL-1**-alpha production and release into the medium. LPS antagonist polymyxin B partly abolished the LPS effect, whereas **IL-1** receptor antagonist (IL-1ra) partly abolished both LPS and mrIL-1 effects. Reversal of LPS-induced effects by IL-1ra was comparable to the reversal of mrIL-1 effects, only the decrease in type II collagen after LPS **treatment** was abolished to a lesser extent by IL-1ra. It is concluded from these results that a considerable part of the matrix degradation by LPS (metalloproteinase activation, proteoglycan loss, type I collagen decrease) is mediated by **IL-1**-alpha, whereas degradation of type II collagen and, partly, reduction of mineralization is induced either directly by LPS or

by mediators other than **IL-1**. **IL-1** is one of the important factors in maintaining **connective tissue** inflammation. Its causative role in matrix degradation, collagen breakdown and inhibition of endochondral mineralization has been shown,using an appropriate model of endochondral mineralization in **vitro**.

ACCESSION NUMBER: 1995:315845 BIOSIS
DOCUMENT NUMBER: PREV199598330145
TITLE: Induction of metalloproteinase activity, cartilage matrix degradation and inhibition of endochondral mineralization in vitro by E. coli lipopolysaccharide is mediated by interleukin 1-alpha.
AUTHOR(S): Scheller, Marina (1); Zimmermann, Bernd; Bernimoulin, Jean-Pierre; Scholz, Peter
CORPORATE SOURCE: (1) Inst. Toxicol. Prenatal Pharmacol., Garystrasse 5, D-14195 Berlin Germany
SOURCE: Cytokine, (1995) Vol. 7, No. 4, pp. 331-337.
ISSN: 1043-4666.
DOCUMENT TYPE: Article
LANGUAGE: English

L8 ANSWER 7 OF 15 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AB Pulpal and periodontal diseases are bacterial infections which result in local **connective tissue** and bone destruction. Effective host resistance to these infections is primarily mediated by neutrophils and other phagocytic cells. PGG glucan (poly-beta 1-6-glucotriosyl-beta 1-3-glucopyranose glucan) is a biological response modifier which stimulates the production of neutrophils and upregulates their phagocytic and bactericidal activity. In the present studies, the effect of PGG glucan on infection-stimulated alveolar bone resorption was tested in an in vivo model. Periapical bone resorption was induced in Sprague-Dawley rats by surgical pulp exposure and subsequent infection from the oral environment. Animals were administered PGG glucan (0.5 mg/kg) or saline (control) subcutaneously the day before and on days 2, 4, 6, 9, 11, 13, 16, and 18 following the pulp exposure procedure. PGG glucan enhanced the number of circulating neutrophils and monocytes and increased neutrophil phagocytic activity approximately two-fold. PGG glucan-**treated** animals had significantly less infection-stimulated periapical bone resorption than control animals, as determined radiographically (-48.0%; $p < 0.001$) and by histomorphometry (-40.8% and -42.4% for first and second molars, respectively; $p < 0.01$). PGG glucan-**treated** animals also had less soft tissue destruction, as indicated by decreased pulpal necrosis. Only 3.3% of first molar pulps from PGG glucan-**treated** animals exhibited complete necrosis, as compared with 40.6% of pulps from controls. Finally, PGG glucan had no effect on either PTH- or **IL-1**-stimulated bone resorption in **vitro**. These findings support the concept that a biological response modifier which enhances endogenous antibacterial mechanisms in neutrophils can decrease infection-stimulated alveolar bone and soft tissue destruction in vivo.

ACCESSION NUMBER: 95:183821 SCISEARCH
THE GENUINE ARTICLE: QK445
TITLE: REDUCTION OF INFECTION-STIMULATED PERIAPICAL BONE-RESORPTION BY THE BIOLOGICAL RESPONSE MODIFIER PGG GLUCAN
AUTHOR: STASHENKO P (Reprint); WANG C Y; RILEY E; WU Y; OSTROFF G; NIEDERMAN R
CORPORATE SOURCE: FORSYTH DENT CTR, DEPT CYTOKINE BIOL, 140 FENWAY, BOSTON, MA, 02115 (Reprint); FORSYTH DENT CTR, DEPT CELL BIOL, BOSTON, MA, 02115; ALPHA BETA TECHNOL INC, WORCESTER, MA, 00000
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF DENTAL RESEARCH, (JAN 1995) Vol. 74, No. 1, pp. 323-330.
ISSN: 0022-0345.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; CLIN
LANGUAGE: ENGLISH
REFERENCE COUNT: 35
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L8 ANSWER 8 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 6

AB The effect of interleukin-1-alpha (IL-1a) and murine epidermal growth factor (EGF) on incorporation of endogenously produced collagenase in the extracellular matrix of soft **connective tissue** was studied in an in **vitro** model system using periosteal explants obtained from rabbit calvariae. Immunohistochemical analysis indicated the highest level of collagenase in explants cultured for 72 hours with **IL-1**-alpha in combination with EGF. Most enzyme appeared to be associated with the extracellular matrix, but labeling was also found in numerous fibroblast-like cells. Explants cultured in the presence of **IL-1**-alpha alone contained less enzyme and in periostea **treated** without cytokines, or with EGF alone, only a faint label, if any, was seen. Freshly isolated, non-cultured periostea contained no detectable enzyme. Extraction of collagenase from periostea revealed that: (1) non-cultured periosteum did not contain detectable levels of enzyme. (2) The amount of total activatable enzyme synergistically increased (10-fold) under the influence of **IL-1**-alpha and EGF, whereas **IL-1**-alpha alone showed a 4-fold enhancement compared to control or EGF-incubated explants. (3) The latent fraction of the enzyme was synergistically increased (up to 100-fold or more) in periostea cultured in the presence of **IL-1**-alpha+EGF (21.17 mU/explant versus 0.05 mU/explant in controls). (4) Active collagenase, on the other hand, appeared to be present in a relatively high concentration in explants cultured without cytokines (2.45 mU/explant versus 0.36 mU/explant in **IL-1**-alpha+EGF-**treated** explants). (5) No enzyme could be extracted from devitalized explants that were incubated in media containing high levels of latent collagenase. It was concluded that collagenase had been incorporated somehow in the extracellular matrix during culturing. In an attempt to analyze breakdown of collagen the amount of hydroxyproline in culture media was assessed. The level of this imino acid released by periostea **treated** without cytokines or with EGF alone was significantly higher than by those incubated with **IL-1**-alpha and EGF. In conclusion, incorporation of collagenase in periosteal **connective tissue** seems to occur during culturing; the level of the incorporated latent enzyme being strongly enhanced by a combination of the cytokines **IL-1**-alpha and EGF. However, as the amount of collagen breakdown had decreased, these findings indicate that **IL-1**-alpha in combination with EGF is able to induce deposition of a large reservoir of latent collagenase in the extracellular matrix, but does not stimulate adequate activation mechanisms to result in enhanced degradation.

ACCESSION NUMBER: 1994:252052 BIOSIS
DOCUMENT NUMBER: PREV199497265052
TITLE: Immunolocalisation of collagenase in rabbit periosteal tissue explants and extraction of the enzyme: The effect of the cytokines IL-1-alpha and EGF.
AUTHOR(S): Van Der Zee, Erwin; Everts, Vincent (1); Hoeben, Kees; Beersten, Wouter
CORPORATE SOURCE: (1) Exp. Oral Biol. Group, Dep. Periodontol., Academic Centre Dentistry, Amsterdam, ACTA Netherlands
SOURCE: Journal of Cell Science, (1994) Vol. 107, No. 4, pp. 1047-1053.
ISSN: 0021-9533.
DOCUMENT TYPE: Article
LANGUAGE: English

L8 ANSWER 9 OF 15 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN

AB Accumulation of hyaluronic acid within the orbital tissues represents a histological hallmark of Graves' ophthalmopathy. The hyaluronic acid/CD44-receptor plays a key role in the binding and metabolism of hyaluronic acid, and affects numerous cellular functions of potential relevance to the pathogenesis of Graves' ophthalmopathy, including cell proliferation, migration, and adhesive interactions between **connective tissue** components and immunocompetent cells. Using a highly sensitive immunoperoxidase technique and monoclonal antibodies directed against the standard CD44 molecule, we examined the expression and modulation of hyaluronic acid/CD44 receptors in cryostat

sections of orbital biopsy specimens derived from patients with severe Graves' ophthalmopathy and normal individuals. Modulation CD44 by cytokines and affinity-purified IgGs derived from patients with Graves' ophthalmopathy was studied in extracts of fibroblast monolayers following stimulation in **vitro**, using SDS-polyacrylamid gel electrophoresis and immunoblotting. Strong immunoreactivity for CD44 was present in all specimens derived from patients with Graves' ophthalmopathy and was detected in fibroblasts residing in retroorbital **connective tissue**, in extracelular matrix components and in mononuclear cell infiltrates. By contrast, in normal orbital specimens, CD44 immunoreactivity was faint and present only in occasional **connective tissue** cells. Recombinant **IL-1**.alpha., TNF.alpha., IGF-1 and GO-IgGs significantly stimulated CD44 expression in Graves' retroocular fibroblasts (range: 168 to 588%; all $p < 0.05$). By contrast, EGF, IL-6, control IgGs and 15% fetal calf serum failed to alter CD44 expression. **Treatment** of monolayers with IFN.gamma. resulted in weak inhibition of CD44 expression. In conclusion, the hyaluronic acid/CD44 receptor is expressed at elevated levels in Graves' orbital **connective tissue** in situ. Certain autocrine/paracrine factors, known to be present in the orbit Graves' ophthalmopathy, as well as patients IgGs, are capable of stimulating the expression of CD44 in Graves' retroocular fibroblasts in **vitro**. Local stimulation of the hyaluronic acid/CD44 receptor and the resulting metabolic and immunological consequences may play a role in the pathogenesis of Graves' ophthalmopathy.

ACCESSION NUMBER: 93154992 EMBASE
DOCUMENT NUMBER: 1993154992
TITLE: [Detection, localization and modulation of hyaluronic acid/CD44 receptor expression in patients with Graves' ophthalmopathy].
NACHWEIS, LOKALISATION UND MODULATION DER HYALURONSAURE/CD44-REZEPTOR-EXPRESSION BEI PATIENTEN MIT ENDOKRINER ORBITOPATHIE.
AUTHOR: Heufelder A.E.; Bahn R.S.; Boergen K.P.; Scriba P.C.
CORPORATE SOURCE: Forschergr. Immunol./Molekularbiol., Medizinische Klinik, Universitat Munchen, Ziemssenstrasse 1,D-8000 Munchen 2, Germany
SOURCE: Medizinische Klinik, (1993) 88/4 (181-184).
ISSN: 0723-5003 CODEN: MEKLA7
COUNTRY: Germany
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 003 Endocrinology
012 Ophthalmology
029 Clinical Biochemistry
037 Drug Literature Index
LANGUAGE: German
SUMMARY LANGUAGE: English; German

L8 ANSWER 10 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 7

AB **Treatment** of vascular endothelial cells with inflammatory cytokines stimulates surface expression of E-selectin (previously known as endothelial-leukocyte adhesion molecule-1) and promotes the transendothelial migration of neutrophils. To asess participation of E-selectin in cytokine-mediated neutrophil migration, an in **vitro** model consisting of monolayers of human umbilical vein endothelial cells (HUVEC) grown on amniotic **connective tissue** was used. When HUVEC-amnion cultures were stimulated for 4 h with relatively low concentrations of **IL-1** (0.1 to 0.15 U/ml), mAb BB11 or H18/7 to E-selectin partially inhibited migration of subsequently added neutrophils. However, when the cultures were stimulated with 15 U/ml of **IL-1** for 4 or 24 h, little to no inhibition was observed. mAb to E-selectin also failed to inhibit migration of neutrophils across HUVEC-amnion cultures **treated** with low doses of **IL-1** when the leukocytes were additionally stimulated by the chemoattractant leukotriene B4. In contrast, migration of neutrophils across **IL-1-treated** HUVEC was profoundly inhibited by mAb to CD11/CD18 leukocytic integrins under all conditions tested. Results of these studies suggest that participation of E-selectin is not essential for migration of neutrophils across

cytokine-stimulated HUVEC in **vitro**; rather, E-selectin can be bypassed in favor of CD11/CD18-dependent mechanisms under appropriate circumstances.

ACCESSION NUMBER: 1992:280786 BIOSIS
DOCUMENT NUMBER: BA94:5436
TITLE: E SELECTIN ENDOTHELIAL-LEUKOCYTE ADHESION MOLECULE-1 IS NOT REQUIRED FOR THE MIGRATION OF NEUTROPHILS ACROSS IL-1-STIMULATED ENDOTHELIUM IN-VITRO.
AUTHOR(S): FURIE M B; BURNS M J; TANCINCO M C A; BENJAMIN C D; LOBB R R
CORPORATE SOURCE: DEP. PATHOL., STATE UNIV. NEW YORK STONY BROOK, STONY BROOK, N.Y. 11794-8691.
SOURCE: J IMMUNOL, (1992) 148 (8), 2395-2404.
CODEN: JOIMA3. ISSN: 0022-1767.
FILE SEGMENT: BA; OLD
LANGUAGE: English

L8 ANSWER 11 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 8
AB Histologic examination of the retroocular **connective tissues** in Graves' ophthalmopathy (GO) reveals lymphocytic infiltration and an accumulation of glycosaminoglycans (GAG), hydrophilic macromolecules produced locally by fibroblasts. We studied in **vitro** effect on fibroblast GAG production of several cytokines and growth factors likely to be secereted by these activated lymphocytes or macrophages. Cultures were established from rectroocular **connective tissue**, extraocular muscle perimysium, and pretibial skin obtained from patients undergoing orbital decompression or eye muscle surgery for severe GO and from normal individuals. Confluent cultures were **treated** with one of the compounds and labeled with [3H]glucosamine or [35S]sulfuric acid for quantitation of [3H]GAG or [35S]GAG accumulation. On the various compounds examined, only interleukin-1 (**IL-1**) and transforming grow the factor (TGF)-.beta. significantly stimulated [3H]GAG accumulation in a dose- and time-dependent fashion. There was no different in sensitivity to the GAG-stimulating effect of **IL-1** or TGF-.beta. between fibroblasts from the four anatomical sites studied or between normal and GO patients fibroblasts. In conclusion, both **IL-1** and TGF-.beta. are potent stimulators of [3H]GAG accumulation by retroocular **connective tissue** and perimysial fibroblasts, as well as by fibroblasts from the dermal sites studied. Stimulation of GAG production by these cytokines, released from lymphocytes or macrophages infiltrating the retroocular space, may play a role in the accumulation of GAG in the rectroocular and perimysial **connective tissues** in GO.

ACCESSION NUMBER: 1992:374776 BIOSIS
DOCUMENT NUMBER: BA94:56826
TITLE: STIMULATION OF GLYCOSAMINOGLYCAN PRODUCTION IN CULTURED HUMAN RETROOCULAR FIBROBLASTS.
AUTHOR(S): KORDUCKI J M; LOFTUS S J; BAHN R S
CORPORATE SOURCE: DIV. ENDOCRINOLOGY, MAYO CLINIC, ROCHESTER, MINN. 55905.
SOURCE: INVEST OPHTHALMOL VISUAL SCI, (1992) 33 (6), 2037-2042.
CODEN: IOVSDA. ISSN: 0146-0404.
FILE SEGMENT: BA; OLD
LANGUAGE: English

L8 ANSWER 12 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 9
AB The anabolic steroid, stanozolol, is used therapeutically to **treat** a number of pathological conditions and its clinical effects suggest that it can modulate **connective tissue** breakdown. The ability of this compound to stimulate prostaglandin E2 (PGE2), collagenase, gelatinase and stromelysin production by human synovial and skin fibroblasts in **vitro** was examined. The results showed that stanozolol significantly stimulated, in a dose dependent manner, PGE2, collagenase and stromelysin production by skin fibroblasts. However, no stimulation was seen in the synovial cell lines. In contrast, no effect on gelatinase production was seen in either cell type, following exposure to stanozolol. The synovial and skin lines both exhibited a significant stimulation of PGE2 and all three metalloproteinases in response to

interleukin-1.beta. (**IL-1**.beta.). The anabolic steroids nortestosterone and oxymetholone demonstrated no ability to stimulate PGE2 or collagenase production in either skin or synovial fibroblasts. These results suggest that stanozolol exerts differential effects on skin and synovial fibroblasts in **vitro** which may enable the elucidation of the mechanism of action of the compound in vivo.

ACCESSION NUMBER: 1992:307781 BIOSIS
DOCUMENT NUMBER: BA94:20931
TITLE: THE DIFFERENTIAL RESPONSES OF HUMAN SKIN AND SYNOVIAL FIBROBLASTS TO STANOZOLOL IN-VITRO PRODUCTION OF PROSTAGLANDIN E-2 AND MATRIX METALLOPROTEINASES.
AUTHOR(S): ELLIS A J; WRIGHT J K; CAWSTON T E; HAZLEMAN B L
CORPORATE SOURCE: RHEUMATOL. RES. UNIT, UNIT E6, ADDENBROOKE'S HOSP., HILLS RD., CAMBRIDGE CB2 1QQ, UK.
SOURCE: AGENTS ACTIONS, (1992) 35 (3-4), 232-237. CODEN: AGACBH. ISSN: 0065-4299.
FILE SEGMENT: BA; OLD
LANGUAGE: English

L8 ANSWER 13 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 10

AB Monocytes were isolated from peripheral blood and cultured in **vitro** for more than 3 weeks in glass chamber slides. Phenotypically and ultrastructurally these nonadherent macrophages (NAM) appear similar to **connective tissue** resident macrophages. They constitutively secrete a high amount of IL-1ra and little or no **IL-1**-alpha or **IL-1** -beta. When exposed to GM-CSF, IL-2, or IFN-gamma for 24 hr, NAM become adherent and undergo dramatic morphological changes. Cytokines **treatment** primes NAM for increased LPS-mediated TNF production and these GM-CSF- and LPS-**treated** NAM are cytotoxic to WEHI 164, a TNF-sensitive target. Morphological changes and TNF production are both inhibited by antimetabolites and a variety of antienoplastic drugs. Although morphology inhibition is reversible under certain circumstances, inhibition of TNF synthesis is irreversible. These findings suggest that cytokines might play a role in differentiation and maturation of long-term cultured monocytes. Furthermore, the effects of antimetabolites and antineoplastic drugs on arresting the differentiation processes may significantly impair antitumor functions of macrophages.

ACCESSION NUMBER: 1993:70770 BIOSIS
DOCUMENT NUMBER: PREV199395035270
TITLE: Cytokine-induced differentiation of cultured nonadherent macrophages.
AUTHOR(S): Tsai, Van (1); Firestein, Gary S. (1); Arend, William; Zvaifler, Nathan J. (1)
CORPORATE SOURCE: (1) Div. Rheumatol., Univ. of California, San Diego, La Jolla, Calif. 92103-8417
SOURCE: Cellular Immunology, (1992) Vol. 144, No. 1, pp. 203-216. ISSN: 0008-8749.
DOCUMENT TYPE: Article
LANGUAGE: English

L8 ANSWER 14 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 11

AB We investigated the effects of several cytokines on HLA-DR expression in cultured fibroblasts derived from retroocular **connective tissue** and pretibial and abdominal skin of patients with Graves' ophthalmopathy (GO) and pretibial dermopathy (PTD), as well as from normal individuals. We hypothesized that differences in response to cytokines between fibroblasts from various anatomical areas might play a role in the site-selective involvement of the extrathyroidal manifestation of Graves' disease. HLA-DR expression in fibroblasts was quantitated by scanning densitometry of whole cell lysates subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. Direct immunofluorescence of cell monolayers was also performed. We hypothesize that unique characteristics of these fibroblasts may play a role in GO and PTD. Cultured retroocular, pretibial, and abdominal fibroblasts from patients with Graves' disease as well as from normal individuals did not express HLA-DR spontaneously. **Treatment** in **vitro** with interferon-.gamma. (IFN.gamma.; 100 U/mL) for 5 days induced HLA-DR by 50-

to 80-fold (P < 0.0001) in fibroblasts from all sites and subjects studied. However, IFN.gamma.-induced HLA-DR expression was significantly greater in retroocular (P < 0.005) and pretibial (P < 0.0005) fibroblasts from patients with GO and PTD than in fibroblasts obtained from the same anatomical sites of normal individuals. Further, retroocular and pretibial fibroblasts from patients with GO and PTD responded to IFN.gamma. more vigorously than did abdominal fibroblasts from these same patients (P < 0.0001). IFN.gamma.-induced HLA-DR expression was enhanced by concomitant **treatment** with tumor necrosis factor-.alpha. (100 U/mL). In contrast, **treatment** of retroocular fibroblasts with transforming growth factor-.beta. (10 ng/mL), epidermal growth factor (1 ng/mL), or interleukin-6 (IL-6; 100 U/mL) significantly attenuated IFN.gamma.-induced HLA-DR reactivity by 40-59% (P < 0.05). Incubation of retroocular fibroblasts with tumor necrosis factor-.alpha., **IL-1** .alpha. (10 U/mL), IL-2 (10 U/mL), IL-6, granulocyte-macrophage colony-stimulating factor (100 U/mL), epidermal growth factor, and transforming growth factor-.beta. alone did not affect HLA-DR expression. These results indicate that several cytokines can influence HLA-DR expression in cultured fibroblasts. The enhanced induction of HLA-DR by IFN.gamma. in retroocular and pretibial fibroblasts compared with that in abdominal fibroblasts may partially explain the selective involvement of the retroocular **connective tissue** and pretibial skin in fully expressed Graves disease.

ACCESSION NUMBER: 1991:430469 BIOSIS
DOCUMENT NUMBER: BA92:86634
TITLE: INCREASED INDUCTION OF HLA-DR BY INTERFERON-GAMMA IN CULTURED FIBROBLASTS DERIVED FROM PATIENTS WITH GRAVES' OPHTHALMOPATHY AND PRETIBIAL DERMOPATHY.
AUTHOR(S): HEUFELDER A E; SMITH T J; GORMAN C A; BAHN R S
CORPORATE SOURCE: DIV. ENDOCRINOL., MAYO CLIN., ROCHESTER, MINN. 55905.
SOURCE: J CLIN ENDOCRINOL METAB, (1991) 73 (2), 307-313.
CODEN: JCEMAZ. ISSN: 0021-972X.
FILE SEGMENT: BA; OLD
LANGUAGE: English

L8 ANSWER 15 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 12

AB The ability of the anabolic steroid, stanozolol, to stimulate procollagenase production by human synovial and skin fibroblasts was examined in an in **vitro** assay system. Stanozolol is used therapeutically to **treat** a variety of **connective tissue** and vascular disorders and its clinical effects suggest that it can modulate **connective tissue** breakdown. The results showed that stanozolol was capable, in a dose dependent manner, of significantly stimulating procollagenase production by skin fibroblasts. However, in three synovial fibroblast lines no evidence was found of increased collagenase production following **treatment** with stanozolol; although the synovial fibroblasts secreted significantly increased amounts of procollagenase in response to **IL-1** . These results may shed some light on the mechanism of action in vivo of stanozolol in the **treatment** of **connective tissue** disorders.

ACCESSION NUMBER: 1990:112851 BIOSIS
DOCUMENT NUMBER: BA89:62342
TITLE: THE EFFECT OF THE ANABOLIC STEROID STANOZOLOL ON THE PRODUCTION OF PROCOLLAGENASE BY HUMAN SYNOVIAL AND SKIN FIBROBLASTS IN-VITRO.
AUTHOR(S): WRIGHT J K; SMITH A J; CAWSTON T E; HAZLEMAN B L
CORPORATE SOURCE: RHEUMATOLOGY RESEARCH UNIT, ADDENBROOKE'S HOSP., HILLS ROAD, CAMBRIDGE, UK.
SOURCE: AGENTS ACTIONS, (1989) 28 (3-4), 279-282.
CODEN: AGACBH. ISSN: 0065-4299.
FILE SEGMENT: BA; OLD
LANGUAGE: English